

201-16310A

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DITP/OSIO**Robust Summary - Polyethylbenzene Bottoms Stream**

2006 JUL 11 AM 9: 20

Fish Acute Toxicity

Test Substance:	Polyethylbenzene Bottoms Stream (PEB) is 100% of the complex mixture CAS RN 68987-42-8. PEB is a coproduct of ethylbenzene manufacture and a Class II complex mixture consisting of various isomers of alkylbenzene and diphenyl hydrocarbons.
Method/guideline:	OECD Guideline 203 (1992)
Type (test type):	Static-renewal, water accommodated fraction
GLP:	yes
Year (study performed):	2006
Species:	fathead minnow (<i>Pimephales promelas</i>)
Analytical Monitoring:	yes
Exposure Period:	96 hours
Statistical Methods:	LC50 by the probit method and the untrimmed Spearman-Kärber method.
Test Conditions: Note: concentration prep., vessel type, volume, replication, water quality parameters, environmental conditions, supplier of organisms, age, size, loading	<p>The PEB sample was a blend of equal volumes of six PEB samples from different suppliers. Groups of fathead minnows were exposed to a negative control, a solvent control (0.05 mL acetone/L), and five concentrations of the test substance for 96 hours. Fish were assessed for mortality and abnormal behavior effects each day. Exposure solutions were prepared as water accommodated fractions (WAFs) of Polyethylbenzene Bottoms (PEB) blend, and exposure solutions were renewed every 24 hours using fresh WAFs. The experimental treatment included control, solvent control, and five PEB loading rates of _____ and 50 mg/L. Dilution water was laboratory freshwater prepared by blending naturally hard well water with well water that was demineralized by reverse osmosis</p> <p>WAFs were prepared by direct addition of 0.0312, 0.0614, 0.123, 0.236, and 0.473 mL volumes of PEB (density = 0.9526 g/mL) to respective 9.5-L glass carboys, each containing 9 L of dilution water. The solvent control carboy and the five carboys containing PEB received 0.450 mL acetone. Each carboy was sealed with parafilm and stirred with a teflon stir bar for approximately 2 hours. Stirring speed was adjusted to create a slight vortex in each bottle (<25% of the solution depth). Once the stirring period ended, liquid phases in the carboys were allowed to separate for approximately 30 min.</p>

Control (dilution water) and solvent control (0.05 mL acetone/L) solutions were treated in the same manner. From each carboy, solution was siphoned into two replicate 3.8-L glass jars, which served as test chambers. The test jars were completely filled such that each chamber held approximately 3.8 L and contained no headspace when jars were sealed with a glass plate. This procedure of test solution preparation was repeated on days 1, 2, and 3. A film was observed on the surface of all test solutions that appeared to increase with increasing concentration.

Fish used in the test originated from established in-house cultures maintained by the testing laboratory. Fish were cultured in the same water as used in testing and at approximately the same temperature. Fish were fed newly hatched brine shrimp (*Artemia* sp.) and a commercial fish food two times a day while in culture. Fish were not fed approximately 48 hours prior to testing or during the test. There were no mortalities in the culture the nine days prior to initiation of the definitive test. Fish selected for the test were approximately two months old and ranged from 22 to 26 mm in total length (mean and standard deviation (SD) = 24 mm \pm 1.5mm) and 0.076 to 0.151 g blotted wet weight (mean and SD = 0.118 \pm 0.0267 g). The loading rate was 0.155 g fish/L of test solution.

Once the test chambers were filled with test solution, fish were distributed one at a time until each test chamber contained its complement of five fish, giving 10 fish per each experimental group. Test chambers were sealed with a glass plate and were not opened except when fish were transferred to newly-prepared WAF solutions at 24, 48, and 72 hours. Test chambers were placed in a temperature-controlled waterbath set to maintain a temperature of 23 \pm 1°C. Fluorescent lighting was maintained on a 16-hour daylight photoperiod with 30-min simulated dawn and dusk periods. Light intensity during the test was 763 lux.

Temperature, dissolved oxygen, and pH were measured in all fresh solutions at 0, 24, 48, and 72 hours, and in all old solutions at 24, 48, 72, and 96 hours. Measurements for dissolved PEB in the test solutions were made on fresh solutions collected at 0 and 72 hours, and on old solutions collected at 24 and 96 hours. Samples were analyzed by gas chromatography (GC) using flame ionization detection (FID). Concentrations of dissolved PEB were calculated from a standard curve as the sum of the responses from six marker peaks within the PEB chromatogram.

Temperature measurements of the exposure solutions during the test ranged from 22.7° C to 23.7° C, dissolved oxygen ranged from 7.9 mg/L to 8.4 mg/L in the new solutions and from 6.2 to 7.2 mg/L in the old solutions. The pH of the fresh solutions ranged from 7.62 to 7.98 and from 7.56 to 7.80 in the old solutions. The dilution water

	<p>at test initiation had a total hardness of 140 mg/L as CaCO₃, total alkalinity of 150 mg/L as CaCO₃, and conductivity of 357 µS.</p> <p>Measured concentrations of PEB in the WAF solutions were:</p> <table><tr><td>Nominal</td><td></td><td></td><td></td><td></td><td></td><td></td></tr><tr><td>Loading Rate, mg/L</td><td>0-hr fresh</td><td>24-hr old</td><td>72-hr fresh</td><td>96-h old</td><td>mean</td><td>% nominal</td></tr><tr><td>Control</td><td><MQL</td><td><MQL</td><td><MQL</td><td><MQL</td><td></td><td></td></tr><tr><td>Solv. Control</td><td><MQL</td><td><MQL</td><td><MQL</td><td><MQL</td><td></td><td></td></tr><tr><td>3.3</td><td>0.795</td><td>0.669</td><td>0.876</td><td>0.792</td><td>0.783</td><td>24</td></tr><tr><td>6.5</td><td>0.943</td><td>0.840</td><td>1.22</td><td>1.13</td><td>1.03</td><td>16</td></tr><tr><td>13</td><td>1.14</td><td>0.945</td><td>1.97</td><td>1.91</td><td>1.49</td><td>11</td></tr><tr><td>25</td><td>1.50</td><td>1.27</td><td>2.27</td><td>2.22</td><td>1.82</td><td>7</td></tr><tr><td>50</td><td>2.82</td><td>2.28</td><td>NS</td><td>NS</td><td>2.55</td><td>5</td></tr></table> <p>Minimum Quantifiable Limit (MQL) = 0.208 mg/L</p>	Nominal							Loading Rate, mg/L	0-hr fresh	24-hr old	72-hr fresh	96-h old	mean	% nominal	Control	<MQL	<MQL	<MQL	<MQL			Solv. Control	<MQL	<MQL	<MQL	<MQL			3.3	0.795	0.669	0.876	0.792	0.783	24	6.5	0.943	0.840	1.22	1.13	1.03	16	13	1.14	0.945	1.97	1.91	1.49	11	25	1.50	1.27	2.27	2.22	1.82	7	50	2.82	2.28	NS	NS	2.55	5
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<p>Results</p> <p>Units/Value:</p> <p>Note: Deviations from protocol or guidelines, analytical method, biological observations, control survival</p>	<p>96-hour LC50 = 1.65 mg/L (95% CL. = 1.43 and 1.87 mg/L), based on mean measured PEB concentrations.</p> <p>96-hour NOEC = 1.03 mg/L, based on mean measured PEB concentrations.</p> <p>96-hour dose-response slope = 14</p> <table><tr><td>Nominal Loading Rate, mg/L</td><td>Mean Measured</td><td>Mean 96-hour % Mortality</td></tr><tr><td>Control</td><td><MQL</td><td>0</td></tr><tr><td>Solv. Control</td><td><MQL</td><td>0</td></tr><tr><td>3.3</td><td>0.783</td><td>0</td></tr><tr><td>6.5</td><td>1.03</td><td>0</td></tr><tr><td>13</td><td>1.49</td><td>30</td></tr><tr><td>25</td><td>1.82</td><td>70</td></tr><tr><td>50</td><td>2.55</td><td>100</td></tr></table> <p>There were no protocol or guideline deviations that adversely affected the study.</p>	Nominal Loading Rate, mg/L	Mean Measured	Mean 96-hour % Mortality	Control	<MQL	0	Solv. Control	<MQL	0	3.3	0.783	0	6.5	1.03	0	13	1.49	30	25	1.82	70	50	2.55	100																																							
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<p>Data Quality</p> <p>Reliabilities</p>	<p>1. Reliable without restrictions</p>																																																															
<p>Reference</p>	<p>Analytical Bio-Chemistry Laboratories (ABC). 2006. Acute toxicity of polyethylbenzene bottoms stream blend (PEB Blend) to the fathead minnow, <i>Pimephales promelas</i>, determined under static-renewal test</p>																																																															

	conditions. ABC study No. 49028, ABC Laboratories, Columbia, Missouri. Sponsor: American Chemistry Council, Arlington, VA.
Other Last changed	